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Racocetra undulata, a new species in the *Glomeromycetes* from Taiwan

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Abstract – A new arbuscular mycorrhizal fungus, *Racocetra undulata* (*Racocetraceae*: *Glomeromycetes*), was found in soil samples collected from the rhizosphere of two coastal plants, *Hibiscus tiliaceus* and *Pandanus odoratissimus* var. *sinensis*, at depths of 0–20 cm in western Taiwan in October 2007. The 195–225 µm broad, globose to subglobose, pale yellow glomerospores, which form terminally on sporogenous cells, have an undulate wall surface and two walls. The germination shields on the outer surface of the inner wall are ovoid, hyaline to subhyaline, with folds separating wavy lobes. The undulate outer spore surface readily differentiates *R. undulata* from all other *Racocetra* species.

Key words – *Gigasporaceae, Glomeromycota, Scutellospora,* taxonomy

Introduction

An unknown species of arbuscular mycorrhizal fungus was found in soil samples collected from the rhizosphere of *Hibiscus tiliaceus* and *Pandanus odoratissimus* var. *sinensis* during an inventory survey of hypogeous fungi in coastal forests of Taiwan in 2007. The species has bi-walled spores on sporogenous cells and a discrete multiply lobed hyaline to subhyaline germination shield on the inner spore wall. Oehl et al. 2008 recently excluded species with such characters from the revised genus *Scutellospora* and transferred them to *Racocetra* in the *Racocetraceae*. Our species, which is readily distinguishable from other *Racocetra* species by the undulate outer spore surface, is described here as a new species, *R. undulata*.

Material & methods

Collection sites, isolation, and pot culture

Soil samples were collected from the rhizosphere of two coastal plants, *Hibiscus tiliaceus* and *Pandanus odoratissimus* var. *sinensis*, at depths of 0–20 cm in the western

region of Taiwan, October 2007. The collection site was situated at 24°41'46"N and 120°51'16"E. The soil was characterized by 0.7–1.1% organic matter, pH (H2O) of 8.2 and 7 $mg.kg^{-1}$ available P (extracted after Mehlich; Nelson et al. 1953). The climate was subtropic with the highest average monthly temperature of 27.7°C, the lowest average monthly temperature of 14.6°C, and the mean annual precipitation of 1500 mm.

Spores were isolated from the soil samples by wet sieving (Gerdemann & Nicolson 1963) and sucrose centrifugation (Jenkins 1964). Spore isolates were suspended in water and illuminated by a quartz-iodine fibre-optic light source. The categorized spores were inoculated with *Sorghum bicolor* in 500 mL pots, each with 30 spores. The pots were filled with autoclaved sand–vermiculite substrate (2:1; w/w) and placed at the greenhouse in the Taiwan Endemic Species Research Institute. The new species has been propagated successfully in pot cultures mixed with *Scutellospora calospora* (T.H. Nicolson & Gerd.) C. Walker & F.E. Sanders.

Morphological analyses

Glomerospores extracted from the pot culture substrates by wet sieving and sucrose centrifugation were mounted in PVLG, PVLG + Melzer's reagent, and H_2O, respectively (Brundrett et al. 1994). Approximately 50 spores were microscopically examined. Terminology follows that introduced for the *Diversisporales* by Oehl et al. (2006), Sieverding & Oehl (2006), and Palenzuela et al. (2008); germination shield structure descriptions follow Walker & Sanders (1986) and Oehl et al. (2008); spore denominations follow Goto & Maia (2006). Permanent slides are curated in Taiwan Endemic Species Research Institute, Chichi, Nantou County, Taiwan (TAIE).

Taxonomy

Racocetra undulata T.C. Lin & C.H. Yen, **sp. nov.** Fig. 1

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Sporocarpia ignota. Sporae singillatim in solo, ad cellulas sporogeneas terminales vel laterales, luteolae, globosae (195–225 µm in diametro) vel subglobosae (180–195 × 190–220 µm); sporae cum tunicis duabus: tunica exterior stratis duabus, stratum exterius luteolum, in solutione Melzeri flavo-aurantium, 1.4–1.7 µm crassum; stratum interius luteolum ad flavum, in solutione Melzeri porphyreum, 2.5–3.7 µm crassum; tunica interior stratis duabus, hyalinibus, stratum exterius 0.7–0.9 µm crassum, stratum interius 1.2–1.5 µm crassum; scutellum germinale in superficie exteriore tunicae interioris, hyalinum ad subhyalinum; ovale vel ellipsoidum, multi-lobatum, 70–85 × 80–90 µm in diametro. Cellulae auxiliares luteolae, singulares, pyriformes vel turbinatae, prominentis nodosis, obtusis.

Type: Glomerospores extracted from Sorghum bicolor pot cultures of spores extracted from soil sample, TAIWAN, Miaoli County, chu-nan Township, 23 Oct. 2007, coll. T.C. Lin. (TAIE, slide no. Ltc 237 – **holotype;** TAIE, slide nos Ltc 238, 239 – **isotypes**).

Etymology: Latin *undulata*, referring to the undulate appearance of the outer spore surface.

Sporocarp Formation – unknown.

Glomerospores singly formed in soils terminally or laterally on a bulbous suspensor cell (= sporogenous cell) (Figs. 1a,d,h). They are pale yellow in color

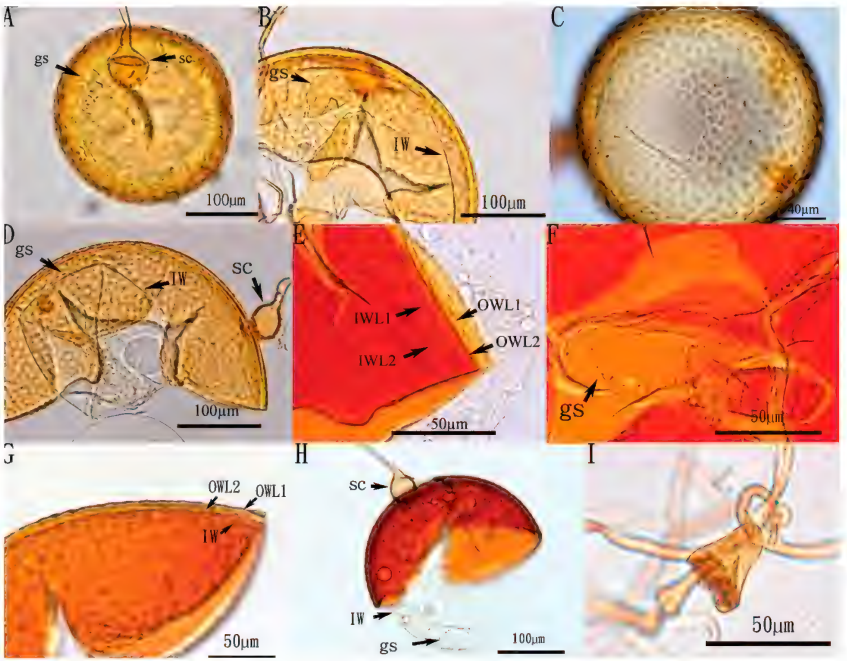

FIG. 1. *Racocetra undulate* A. Spore with sporogenous cell (sc) attached. B. Germination shield (gs) with wavy lobes and smooth margin. C. Uncrushed spore with undulate appearance. D. Hyaline germination shield on the surface of inner wall (IW) in cross view. E. Spore wall structure with two-layered outer wall (OWL1-2) and two-layered inner wall (IWL1-2), OWL1 with yellow-orange reaction and OWL2 with orange-brown reaction in Melzer's reagent. F. Lobes of germination shield forming nicked margins by shallow incisions. G. (IWL1) is closely adherent to (IWL2) and difficult to observe. H. IW and germination shield have no reaction in Melzer's reagent. I. Auxiliary cell formed individually on coiled hyphae with knobby projection on the upside).

and may turn (yellow-orange to) orange-brown in PVLG + Melzer's reagent (FIGS. 1A–D). They are globose (195–225 μm in diameter) to subglobose (180–195 × 190–220 μm) and have two walls: an outer wall and an inner wall (OW and IW; FIGS. 1E,G).

OUTER WALL is two-layered. The outermost wall (OWL1) is about 1.4–1.7 μm thick, with regular undulated thickenings (FIGS. 1C,E), pale yellow in color, but yellow-orange after staining with Melzer's reagent (FIGS. 1E,G). The second layer (OWL2) is 2.5–3.7 μm thick, pale yellow to yellow in color, orange-brown after staining with Melzer's reagent. (FIG. 1E).

INNER WALL is two-layered, bearing a germination shield on the outer surface (FIGS. 1B,D,E). The outer layer of the inner wall (IWL1) is hyaline, flexible, and 0.7–0.9 μm thick, closely adherent to second layer (IWL2) that is

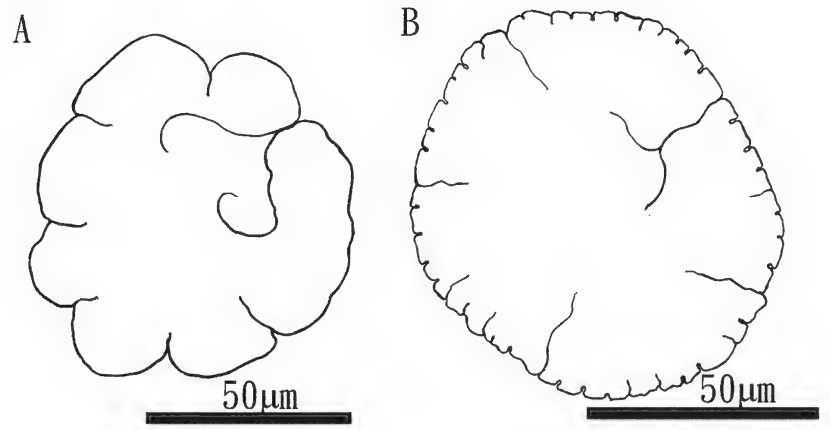

FIG. 2. *Racocetra undulata*: drawings of multiply lobed, wavy germination shields in planar view. A. Germination shield with wavy lobes and smooth margins in the early stage of shield formation. B. Lobes of germination shield forming with nicked margins by shallow incisions (dentate folds) as the shield grows and differentiates further.

hyaline, flexible, and 1.2–1.5 μm thick. The two layers do not react to Melzer's reagent (FIG. 1H).

SPOROGENOUS CELL is globose to elongate, concolorous with the spore, 37–43 μm long and 33–38 μm broad. The wall is composed of two layers that are contiguous with OWL1 and OWL2. OWL1 on the sporogenous cell is about 0.6–0.7 μm, and the adherent OWL2 is about 1.4–1.6 μm thick. The sporogenous hypha is concolorous with the spore wall, 6.5–10.5 μm in diameter, and has the wall of 1.4–1.6 μm thick.

GERMINATION SHIELD on the outer surface of the inner wall is oval to ellipsoid (70–85 × 80–90 μm), hyaline to subhyaline, and has 4–6(–8) wavy lobes, which are separated by folds. The lobes have smooth margins in the early stage of shield formation (FIG. 1B, 2A), but they form nicked margin by shallow incisions (FIG. 1F, 2B) as the shield grows and differentiates further.

AUXILIARY CELLS are 36–41 μm long and 29–33 μm broad and often forms individually on closely spaced branches of coiled hyaline hyphae that are 3–4 μm broad; each cell with a thin wall (<1 μm thick), pale yellow in transmitted light, pyriform to turbinate, and an almost smooth surface, that has, however, 3–4 blunt, knobby projections on the surface (FIG. 1I).

MYCORRHIZA FORMATION is unknown. Attempts in pure culture failed, though the species sporulated in pot culture on *Sorghum bicolor*, together with *Scutellospora calospora*.

Distribution: Glomerospores of this species have been collected only from coastal sand dunes in the western region of Taiwan. The sites were dominated by *Hibiscus tiliaceus* L. (*Malvaceae*) and *Pandanus odoratissimus* var. *sinensis* (Warb.) Kaneh. (*Pandanaceae*) in sandy soils with very low phosphorus content.

Discussion

During the recent revision of *Scutellospora,* species characterized by bi-walled spores and multiply lobed, hyaline to subhyaline germination shields on the inner wall outer surfaces were transferred to a new genus and family, *Racocetra* (*Racocetraceae*) (Oehl et al. 2008). The undulate outer spore surface readily distinguishes our new species, *R. undulata,* from other *Racocetra* species. The other known *Racocetra* species produce spines, warts, or projections on the outer surface, e.g., *R. beninensis* Oehl et al. (Tchabi et al. 2009), *R. coralloidea* (Trappe et al.) Oehl et al. (Gerdemann & Trappe 1974), *R. gregaria* (N.C. Schenck & T.H. Nicolson) Oehl et al. (Nicolson & Schenck 1979), *R. minuta* (Ferrer & R.A. Herrera) Oehl et al. (Ferrer & Herrera 1981), *R. persica* (Koske & C. Walker) Oehl et al. (Koske & Walker 1985), and *R. verrucosa* (Koske & C. Walker) Oehl et al. (Koske & Walker 1985).

The spores of *R. fulgida* (Koske & C. Walker) Oehl et al. (Koske & Walker 1986) resemble *R. undulata* in size and color but have a smooth surface, which easily differentiates the two when their spores are crushed in Melzer's reagent. Although the inner wall of both species does not react with Melzer's reagent, the owl2 of *R. undulata* becomes range-brown, while that of *R. fulgida* turns bright yellow.

Juvenile spores of *R. undulata* closely resemble mature spores of *S. calospora* with pale yellow pigmentation and spore diameter range. However, *S. calospora* spores have a smooth outer surface, unlike the undulate outer spore in *R. undulata.*

R. undulata sporulated in pot cultures with *Sorghum bicolor*, though not abundantly. Despite repeated attempts, we have been unable to establish single-species pot culture of the new species.

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